New Methodology for the synthesis of chloramphenicol

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12/15/2016

Abstract:

For this project, we attempted a new synthesis of Chloramphenicol, An antibiotic used that inhibits the protein synthesis in bacteria. This involves a series of reactions, some of which were to create materials which were unavailable to us, and some of which were essential to the production of the compound. First, we carried out an acylation reaction between Dichloroacetyl Chloride and Aminoacetaldehyde dimethyl acetal with triethylamine to act as a base and ethyl acetate as a solvent. Then, that product underwent a hydrolysis using THF and 2M HCl to convert the acetal to an aldehyde which went in about 75% yield. L-proline was then used to catalyze a reaction in conjunction with DMSO to add 1 equivalent of P-nitrobenzaldehyde to the aldehyde product, which should result in one hydroxyl group facing forward and another one facing backward. L-proline acts to add the hydroxyl groups in a stereochemically-specific way, which should be the D-threo conformation. That reaction was based on a publishing in the Journal of the American Chemical Society by Carlos F. Barbas III, et al. The next reaction involved reduction by Sodium Borohydride to reduce the aldehyde to the corresponding alcohol.

Introduction:

Chloramphenicol has been previously industrially synthesized by means of a nitro-aldol reaction. Reacting nitro ethanol with base makes an anion (potentially explosive) which attacks the aldehyde carbon. Following reduction of the amide to the amine. Both Enantiomers are produced and a resolution with a chiral acid will have be performed to isolate the desired enantiomer. Dr. Lavey has proposed an alternate route for synthesis. This has the potential to cut 4-5 steps from traditional methods of production. We are also trying to synthesize chloramphenicol more efficiently while avoiding the use of any toxic or potentially explosive

compounds. Chloramphenicol was first isolated from cultures of Streptomyces venezuelae in 1947 but now produced synthetically. The compound has 2 diastereomers: The D-threo isomer contains antibacterial activity and L-erythro has little antibacterial activity. Acylation of hydroxyl group inactivates the drug and removal of the dichloroacetamide side chain eliminates biological activity. L-Proline determines the chirality of the products, preventing a low decrease in yield. Chloramphenicol has a melting point range of 148-152 ˚C.

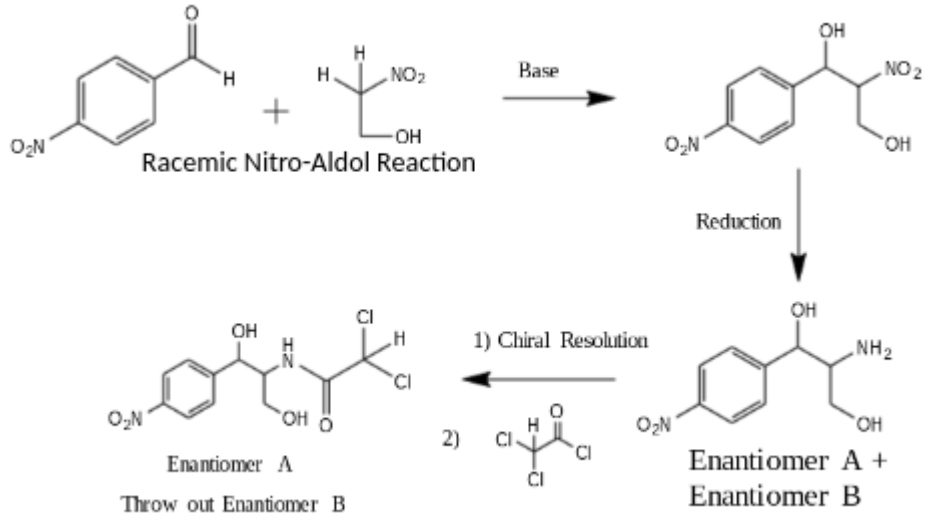


Figure one: Industrial synthesis of Chloramphenicol

Experimental section:

Instrumentation and Materials (All materials used for preparation were reagent grade):

- NMR spectra were recorded using NMRReady™ 60 Nanalysis Corporation.

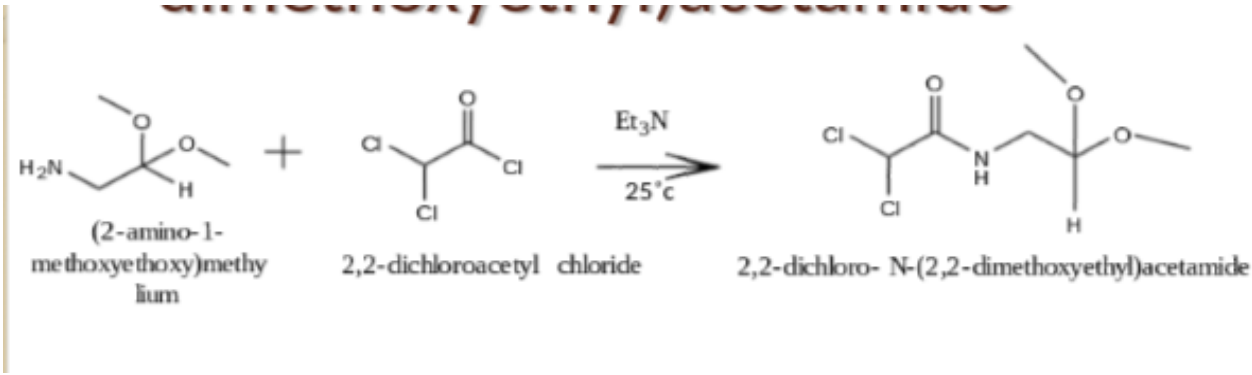
- $CDCL_3$, D_2O and Acetonitrile-D3 were used as references/ blanks and also as solvents for all compounds during the NMR.
- Rotary Evaporator
- Small Column for Flash Chromatography
- TLC Chamber and Plates

Reagents:

- Aminoacetaldehyde Dimethyl Acetal
- Dichloroacetyl chloride
- L-Proline
- P-nitrobenzaldehyde
- Triethylamine
- Sodium Borohydride
- Ethyl Acetate
- N-hexanes
- Tetrahydrofuran (THF)
- 2M HCl
- DMSO

First Experiment:

Step one: Acylation Reaction



- In a 150 mL round-bottomed flask add the following reagents: 1 mMoles of Aminoacetaldehyde dimethyl acetal, 3 mL THF, 1 mMoles triethylamine and 1 mMoles of Dichloroacetyl chloride.
- Add a micro stir bar to the mixture.
- Cover the round-bottomed flask with a glass stopper and let stir overnight.

Figure 2: Reaction flask after stirring overnight

- Compound appeared as a clear yellow oil.

Characterization of Di-acetal Product by NMR:

- The obtained NMR was compared to a predicted NMR produced by the software Chemdraw.

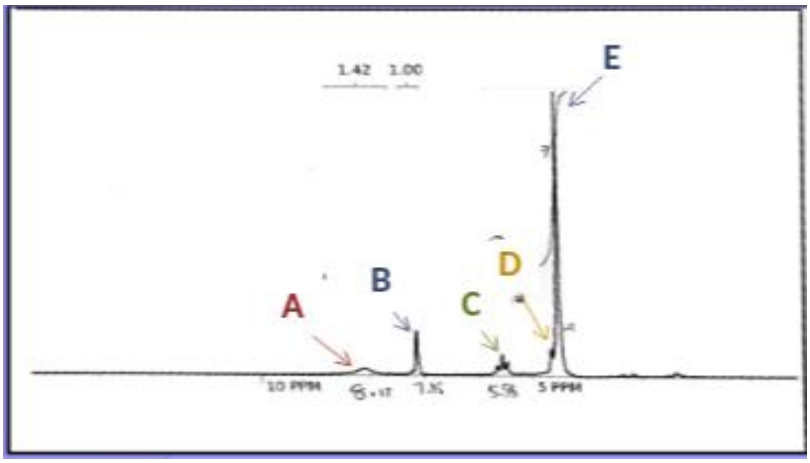


Figure 3: NMR spectrum of 2,2-dichloro-N-(2,2-dimethoxyethyl)acetamide in solvent CDCl3.

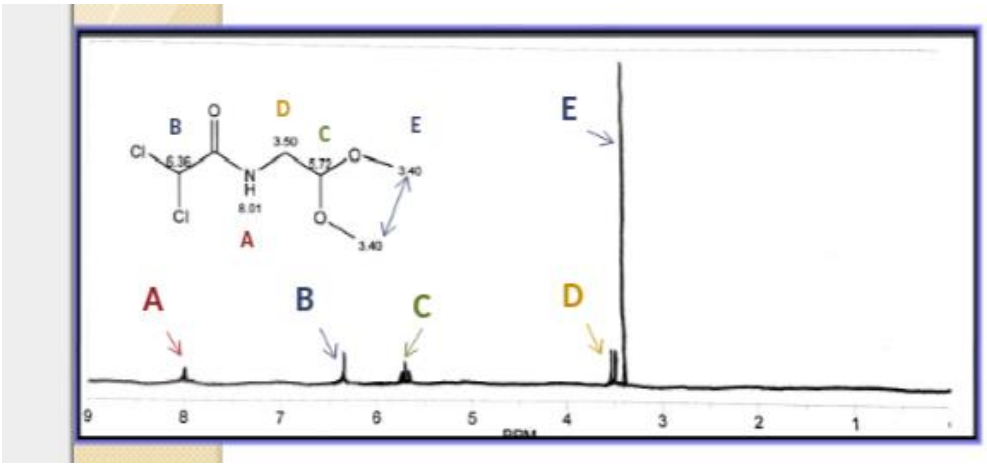
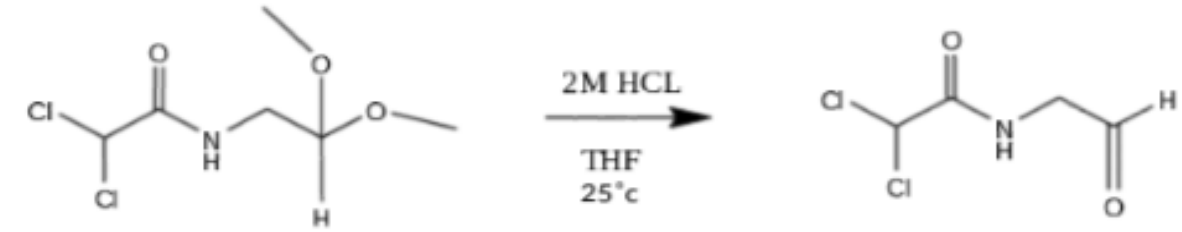


Figure 4: Predicted NMR spectrum taken by Chemdraw in solvent DMSO.

- The difference in the chemical shifts of the peaks is due to the different NMR solvents.
- After the NMR is obtained, Workup reaction and extract product.
- Quench with NH_4Cl. Wash three times with ethyl acetate.
- The compound then is dehydrated with Sodium sulfate and taken to the Rotovap for removal of the solvent at 45℃.
- Vacuum pump for at least 15 minutes.

Step two: Hydrolysis of the di-acetal group



- Add 2 mL HCL followed by 2 mL THF.
- Let reaction stir overnight
- Neutralize reaction mixture with $NaHCO_3$ and check with pH paper.
- Work up with 20 mL brine and 3x15 mL of EtOAc

Figure 5: Separation of organic layer by workup procedure

Characterization of Aldehyde Product by NMR:

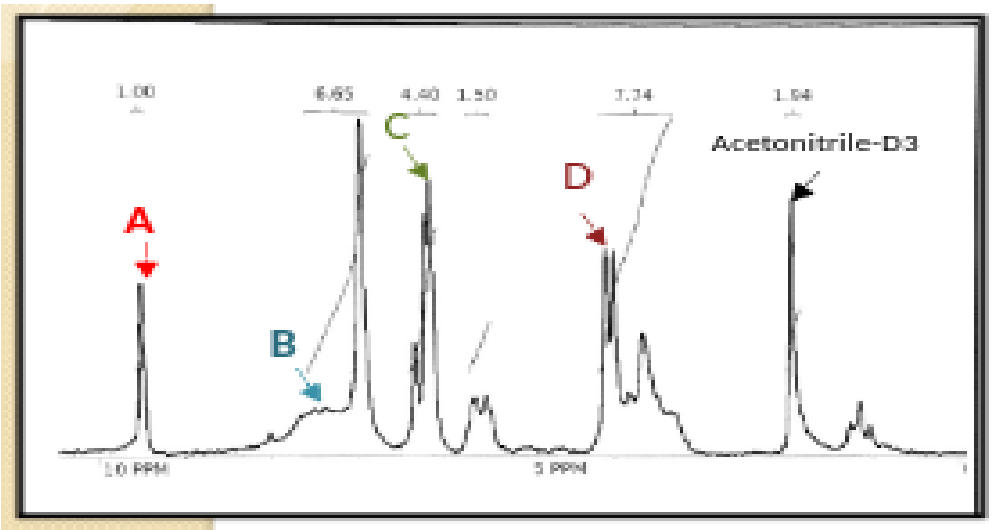


Figure 6: NMR spectrum of aldehyde product taken in Acetonitrile-D3 solvent

- The other peaks were obtained from the solvent ethyl acetate that hasn't been dried completely.

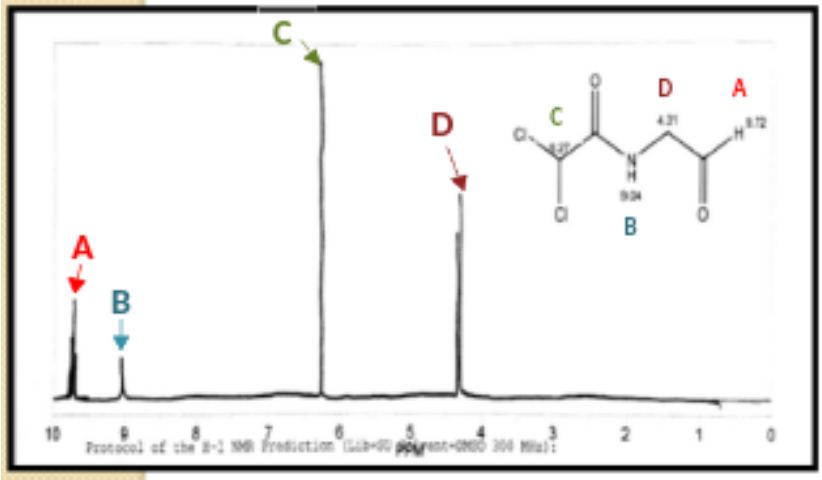


Figure 7: Predicted NMR spectrum taken by Chemdraw software in DMSO

- The difference in the chemical shifts of the peaks is due to the different NMR solvents.
- The desired Aldehyde peak was obtained between 9-10 ppm.

- The pure product is then isolated by column chromatography and monitored by TLC.
- Note: The following compound did not contain chromophore, it can not be seen under UV light.

Figure 8: Isolation of pure aldehyde compound

- A silica column was used to purify the aldehyde compound before starting the L-Proline catalyzed reaction.

- The solvent mixture was determined by the TLC solvent which gave the desired RF value of 0.2.

- 20 fractions were collected from the column and spotted on a TLC plate.

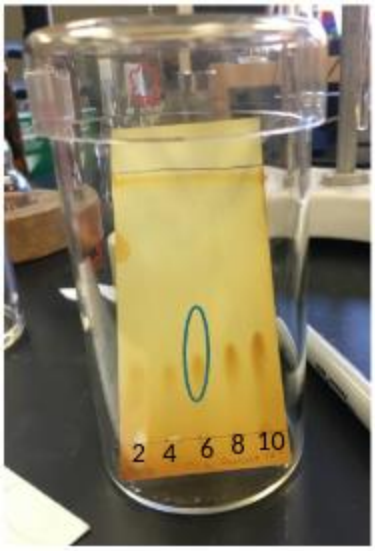
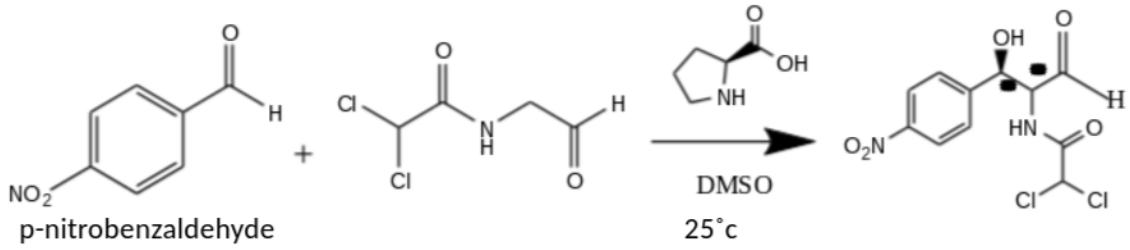


Figure 9: TLC plate in iodine chamber

- The compound appeared in fractions 6-19 and was then collected and dried from the solvent by means of the Rotovap at 40˚C.

Second Experiment: Synthesis of Chloramphenicol

Step one: L-Proline Catalyzed Aldol Reaction



- Add 1 equivalence of L- Proline and 1 equivalence of P-Nitrobenzaldehyde

- 4 mL DMSO (solvent) were added to the reaction flask.
- Leave reaction to stir for several hours and follow up with a routine workup to quench the reaction, neutralize the acid and remove any inorganic salts using brine.
- Evaporate solvent by means of Rotovap at 45˚C.

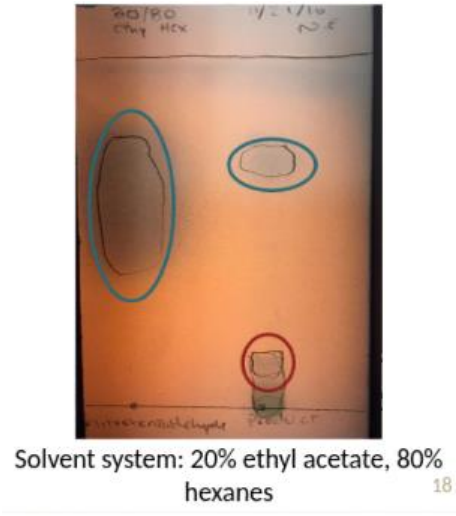


Figure 10: TLC of starting material vs. L-proline catalyzed product

(Note: Compound is now UV active)

Characterization of Cyclic Product by NMR:

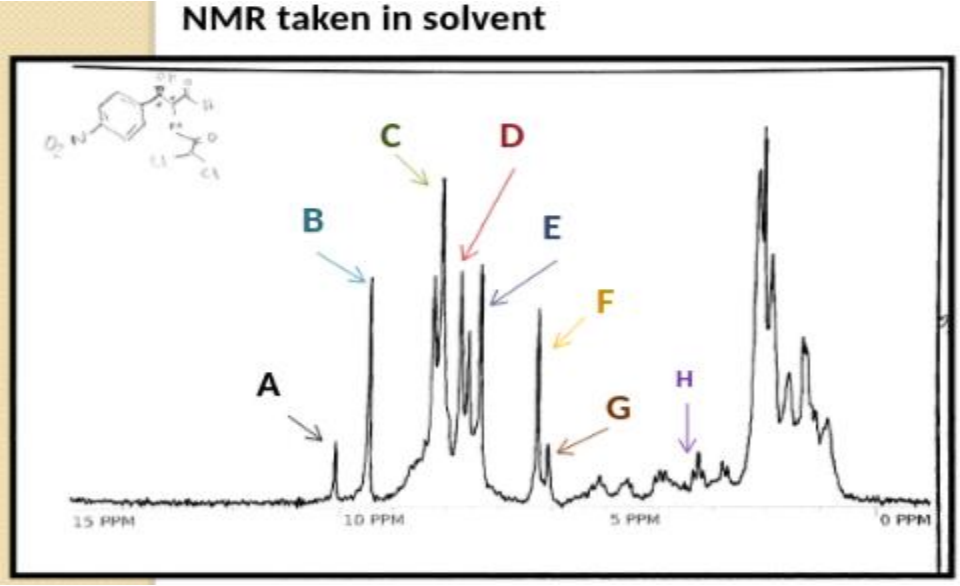


Figure 11: obtained NMR spectrum of Cyclic product

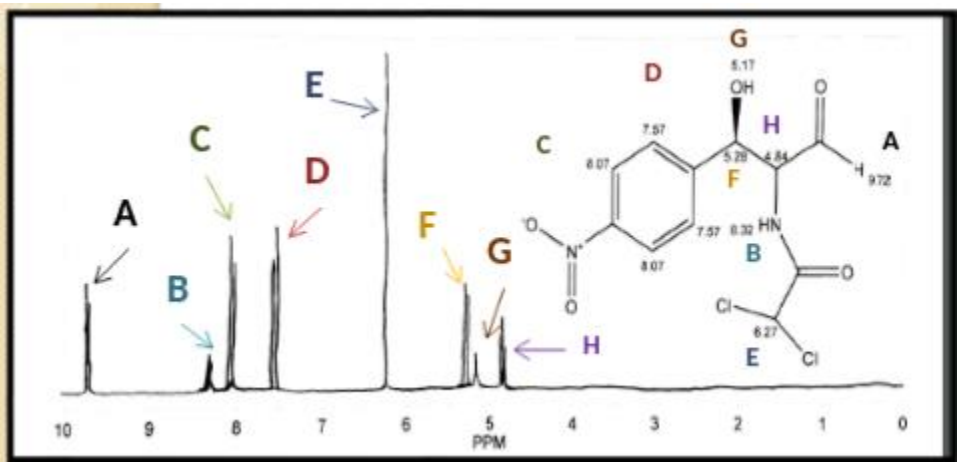
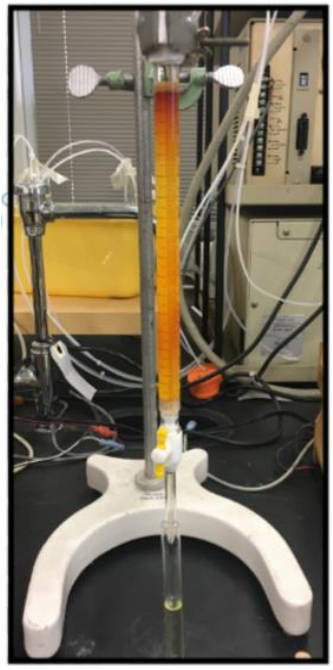


Figure 12: Predicted NMR spectrum taken by Chemdraw software in DMSO

- AB pattern in now visible in the NMR spectrum for the benzene ring.
- Results also show formation of by-products.



10% Ethyl acetate/ 90% H*Figure 13: Isolation of product prior to reduction*

- Separation of pure compound from by products must be done by column chromatography to purify the compound.
- Start by running a 20% solution down the column then increase to 25% then, finally 30% until the desired product stops appearing on the TLC plate.
- Collected a total of 50 fractions.
- Product appeared in fractions 16-32.

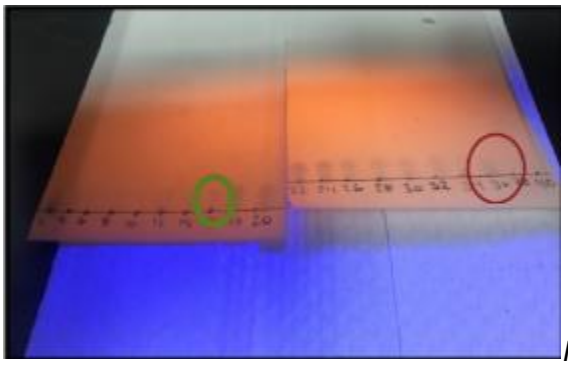
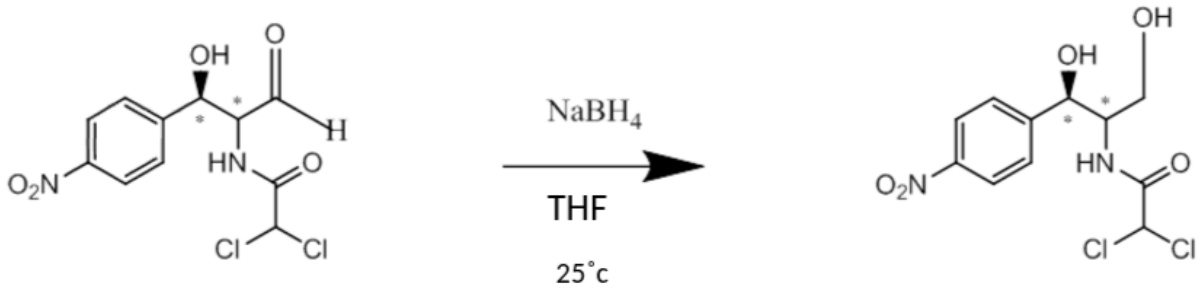


Figure 14: Monitored fractions by TLC

- Fractions are collected and solvent is rotovapped.
- Leave compound under vacuum for at least 15 mins.

Step Two: Sodium Borohydride Reduction



- 1 equivalent of sodium borohydride was added with 4 mL of THF.
- The reaction was left to stir overnight.
- Followed by a routine workup and rotovap at 45˚C.
- NMR of the final product is being taken.

Conclusion:

In summary, we have completed a series of reactions to create Chloramphenicol. The L-Proline catalyzed reaction NMR shows that the desired product is believed to be synthesized. Determination of a pure product will be obtained by taking the melting point. Testing of the antibiotic may also be done by plating a culture of bacteria. Future reactions should go much smoother now that the guidelines have been laid out.

Acknowledgment:

- Dr. Phalguni Ghosh
- Dr. Brian Lavey
- Dr. Steven Rowley
- Dr. Parag Muley, Chairperson , Natural Sciences
- Linda Scherr , Dean, Natural Sciences
- Bristol-Myers Squibb for financial support
- Douglas Reardon and David Rossen, Lab coordinators

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